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A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes

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This article is the result of an initiative between the European Federation of Pharmaceutical Industries Associations (EFPIA) and the European Centre for the Validation of Alternative Methods (ECVAM). Its objectives are to provide the researcher in the safety evaluation laboratory with an up-to-date, easy-to-use set of data sheets to aid in the study design process whilst at the same time affording maximum welfare considerations to the experimental animals.

Although this article is targeted at researchers in the European Pharmaceutical Industry, it is considered that the principles underpinning the data sets and refinement proposals are equally applicable to all those who use these techniques on animals in their research, whether in research institutes, universities or other sectors of industry. The implications of this article may lead to discussion with regulators, such as those responsible for pharmacopoeial testing.

There are numerous publications dealing with the administration of test substances and the removal of blood samples, and many laboratories also have their own 'in-house' guidelines that have been developed by custom and practice over many years. Within European Union Directive 86/609EEC[1] we have an obligation to refine experiments to cause the minimum amount of stress. We hope that this article will provide background data useful toduplication of animal work, as well as sharing practical skills variety of scie

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The objectives of the Technical Sub group of EFPIA/ECVAM were as follows:

(i) to provide a guide on administration volumes for use in common laboratory species used in toxicity studies required by regulatory authorities;
(ii) to provide consensus dosage levels for routine use that represent good practice in terms of animal welfare and practicality;
(iii) to produce a guide to dosage levels representing the upper limit of common practice, which leaves scope to make the case for special investigations.

Administration volumes

Table 1 presents administration volumes for the commonly employed routes in the most frequently used species. They are consensus figures based on published literature and internal guidelines. The marmoset and minipig are now considered within this category because they are being used increasingly in Europe.

Two sets of values are shown in each column: values on the left are intended as a guide to 'good practice' dose volumes for single or multiple dosing; values on the right, where given, are the possible maximal values. If maximal values are exceeded, animal welfare or scientific implications may result and reference to the responsible veterinary surgeon should be made. In some instances values are there to accommodate pharmacopoeial requirements.

Some of these suggested maximum values have been obtained from recent literature,[3,4] but appear high when compared with 'good practice' values. The need for careful attention to animal welfare and the formulation of material used at high dose volumes are rehe nee0o6-3797re'materify

Table 6. Summary of the advantages and disadvantages of the various methods of blood sampling

Ro ı e/ ein	General anae ı he ia	Ti e damage[a]	Repea bleed	Vol me	Specie
J g lar	No	Lo	Ye	+++	Ra , dog, rabbi
Cephalic	No	Lo	Ye	+++	Macaq e, dog
Sapheno /la eral ı ar al	No	Lo	Ye	++(+)	Mo e/ra , marmo e /macaq e, dog
Marginal ear	No (local)	Lo	Ye	++ +	Rabbi , minipig
Femoral	No	Lo	Ye	+++	Marmo e /macaq e
S bling al	Ye	Lo	Ye	+++	Ra
La eral ı ail	No	Lo	Ye	++(+) +	Ra , mo e/marmo e
Cen ı ral ear a ı er	No (local)	Lo	Ye	+++	Rabbi
Cranial ena ca a	No	Lo	Ye	+++	Minipig
Tail ı ip amp ı a ion (<1 3 mm)	Ye	Mod	Limi ed	+	Mo e/ra
Re rob lbar ple	Ye	Modera e/high	Ye	+++	Mo e/ra
Cardiac[b]	Ye	Modera e	No	+++	Mo e/ra /rabbi

[a]The po en ial for ı i e damage i ba ed on ı he likel incidence of i occ rring and ı he e eri of an eq elae, e.g. in amma or reac ion or hi ı ological damage.

[b]

removing the clot. Serial amputations resulting in a significant shortening of the tail (i.e. >5 mm) are not acceptable. The technique may not be suitable for older animals. Anaesthesia is recommended.

Cardiac puncture. This should always be carried out under general anaesthesia and in the past it has been used with recovery in small rodents due to the lack of alternative routes. However, other methods are now available and because of potentially painful and fatal sequelae, such as pericardial bleeding and cardiac tamponade, this technique should only be used for terminal bleeds.

Retrobulbar plexus. The retrobulbar route has been commonly used by researchers in the past but has been observed to cause adverse effects. Concern has therefore arisen because of these effects and their potential severity. Recently, however, other methods have been developed that meet the scientific requirements and also improve the welfare of the animals. Nevertheless, the Technical Subgroup felt that it was worth reviewing in detail some of the advantages and disadvantages of retrobulbar bleeding in the context of the new methods.

Bleeding from this plexus always should be carried out under general anaesthesia in all species and anaesthesia is a requirement in some national regulations. The method has been described in detail by a number of workers.[46–48]

There is little published work on refining the method. The approach (lateral or access via the dorsal or upper aspect of the eye in rats) as the optimal way to penetrate the conjunctiva in order to minimize tissue damage has been discussed.[23] An interval of 2 weeks between bleeds at the same site should allow damaged tissue to repair in most cases,

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